

Wave Effects on the Vertical Migration of Two Benthic Microalgae: *Hantzschia virgata* var. *intermedia* and *Euglena proxima*

MICHAEL B. KINGSTON^{1,2}

Duke University Marine Laboratory
Beaufort, North Carolina 28516-9721, USA

AND

Department of Botany
Duke University
Durham, North Carolina 27708, USA

ABSTRACT: Two benthic microalgal species, *Euglena proxima* (Dangeard) and the diatom *Hantzschia virgata* var. *intermedia* (Grun) Round, congregated at the sediment surface of intertidal sand flats surrounding the Duke University Marine Laboratory in Beaufort, North Carolina, during low tide and migrated below the surface prior to tidal flooding. *E. proxima* was found down to a depth of 12 cm below the surface during high tide while *H. virgata* was not found below 8 cm. One potential advantage of vertical migration for benthic microalgae is avoidance of the sediment surface during high tide when the winnowing action of waves can remove cells from the intertidal habitat. This hypothesis was tested by comparing the mean depths of *E. proxima* and *H. virgata* exposed to calm water conditions with their mean depths under wave-exposed conditions in shallow outdoor tidal tanks equipped with wave generators. Two sediment treatments were tested in each tank to evaluate the effect of a subsurface layer of black sediment (subsurface sediments, presumably reduced) on vertical migration: a uniform column of light brown sand (surface sediments; presumably oxygenated), and a layered column with light brown sand overlying a layer of black sediment. One-way and two-way ANOVAs indicated that wave action increased the mean depth of both species under certain conditions related to the presence, absence, and depth of a subsurface layer of black sediment. In an experiment with a shallow black layer (5 mm), wave action did not result in a significant increase in the mean depth of *H. virgata* as it did in a previous experiment with a deeper black layer (3 cm). Unlike *H. virgata*, *E. proxima* exhibited a wave effect only in the absence of a subsurface black layer. A subsurface black layer seemed to reinforce downward migration in *E. proxima* under both calm water and wave conditions. The absence of a wave effect in the presence of a subsurface black layer suggests that the mean depth of *E. proxima* during high tide was more tightly controlled by the depth of the black layer than wave energy.

Introduction

The vertical migration of organisms in the water column is an almost universal phenomenon in the marine environment—fish (Neilson and Perry 1990), crustaceans (Forward 1976, 1988), cnidarians (Anderson et al. 1992), salps (Angel 1989; Anderson et al. 1992), and phytoplankton (Forward 1976) undergo daily vertical migrations. The marine environment is dominated by vertical gradients in light, temperature, nutrients, food availability, and predation pressure (Angel 1985). Vertical migration may enhance survival in this stratified environment. Organisms that remain in deeper water during the day and then rise to the surface at night are able to take advantage of abundant food supplies in the surface waters at night when the predation efficiency of visual hunters is reduced (Gliwicz 1986; Bollens and Frost 1991).

Vertical migration is not restricted to the water column; it also occurs in marine and estuarine sediments on a much smaller scale (Palmer 1976; Joint et al. 1982). The vertical migration of benthic diatoms to form colored patches on intertidal flats during low tide was first reported by Fauvel and Bohn in 1907. Subsequent research has revealed that this behavior occurs in a number of benthic microalgal taxa, including cyanobacteria, dinoflagellates, euglenoids, diatoms, and a chrysomonad (Palmer and Round 1967; Palmer 1976).

The maintenance of vertical migration by microalgae inhabiting sand flats is most likely related to the costs and benefits associated with an epibenthic existence during different phases of the tidal cycle. The typical pattern of migration results in residence near the sediment surface during daytime low tide and residence deeper within the sediment during daytime high tide and throughout the night (Aleem 1950; Callame and Debyser 1954; Palmer and Round 1965, 1967; Hopkins 1966a; Round and Palmer 1966). During summer daytime low tides, benthic microalgae on the sediment sur-

¹ Corresponding author; tele: 336/584-2412; fax: 336/538-2643; e-mail: Kingston@numen.elon.edu.

² Present Address: Campus Box 2142, Elon College, Elon College, North Carolina 27244-2020.

face experience high light levels, high temperature, desiccation, and disturbance by fiddler crabs (Taylor 1967). During high tides, microalgae on the sediment surface are subjected to wave disturbance and predation by the mud snail *Ilyanassa obsoleta* (Fenchel and Kofoed 1976; Connor 1980; Connor et al. 1982). Possible advantages to periodic migration below the surface include escape from surface predators, avoidance of wave disturbance, and access to subsurface nutrients.

One of the most constant and predictable selective pressures experienced by benthic microalgal populations in the intertidal zone is the removal of cells by the winnowing action of waves (Hopkins 1966b). Tidal resuspension of intertidal mud flat sediments and their associated epibenthic flora results in a net transport of algae from the mud flat to the salt marsh where they are more likely to be ingested by filter feeders (Baillie and Welsh 1980). A census of the microalgal species found on intertidal sand flats near the Duke University Marine Laboratory, Beaufort, North Carolina, showed that only those species displaying vertical migration attained densities exceeding 10^5 cells cm^{-2} while nonmigrating species seldom exceeded 10^2 cells cm^{-2} (Kingston 1990). This large difference between migrating and nonmigrating species suggests migratory behavior may contribute to the maintenance of high population densities. The descent of the community before tidal inundation and its ascent post-inundation to the sediment surface would prevent exposure of the population to the daily winnowing action of waves at the sediment surface (Hopkins 1966b).

The idea that downward migration is an escape from wave disturbance is not a new one (Fauré-Fremiet 1951; Ganapati et al. 1959; Heckman 1985); yet, the importance of waves in the maintenance of vertical migration behavior has never been tested experimentally. Previously, only anecdotal observations in the field and laboratory have been used to support this hypothesis (Perkins 1960; Hopkins 1963, 1966b; Palmer and Round 1967).

The present study utilized outdoor tidal tanks equipped with wave generators to evaluate the effect of wave motion on the vertical distribution of the diatom *Hantzschia virgata* var. *intermedia* (Grun) Round and *Euglena proxima* (Dangeard) at high tide. It was hypothesized that the mean depth of populations subjected to waves would be deeper than that of populations not subjected to wave action. These two species were chosen because they form large, monospecific patches on the sand flats surrounding the Duke University Marine Laboratory. Additionally, the influence of a subsurface layer of black sediment on the response of the pop-

ulations to wave motion was examined. This sediment treatment was included because the ability to tolerate hypoxic and reducing conditions like those in the black sediment layer has been implicated in the horizontal distribution of mud flat diatoms (Hopkins 1964; Admiraal and Peletier 1979) and is likely to influence vertical distributions as well.

Materials and Methods

STUDY LOCATION

This study was conducted at the Duke University Marine Laboratory, Beaufort, North Carolina, USA. From late September through late July, *Euglena proxima* (Dangeard) formed large visible patches during daytime low tides on the intertidal sand flats surrounding the laboratory (Kingston 1990). *Hantzschia virgata* var. *intermedia* (Grun) Round patches were observed only during the warmer months—late May through mid-October. In addition to seasonal differences, these two species displayed markedly different tidal height distributions; *E. proxima* patches were located in the upper intertidal zone between 0.55 m and 0.67 m above mean low water (MLW), while *H. virgata* was found in the lower intertidal between 0.15 m and 0.34 m above MLW.

In lower intertidal areas where *H. virgata* patches were found, black sediment was overlain by a very thin layer of lighter colored sediment 2–6 mm in thickness. This black sediment is indicative of anaerobic decomposition and a redox-potential-discontinuity (Reise 1985). Since neither oxygen nor redox potentials were measured in this study, Reise's (1985) terminology will be adopted here; the lighter colored shallow sediment will be identified as the yellow layer and the subsurface black sediment will be termed the black layer. In the upper intertidal areas where *E. proxima* was located, the depth of the yellow layer varied from 2 cm to 10 cm thick depending upon the season and tidal height (Kingston 1990).

IN SITU VERTICAL DISTRIBUTIONS

In situ vertical distribution profiles of *H. virgata* were determined by collecting 8-cm-deep cores from microalgal patches on the intertidal sand flat adjacent to the Duke Marine Lab's dock in July 1993. Preliminary sampling revealed that deeper cores (12 cm) were required in October 1993 to adequately determine the vertical distribution of *E. proxima* since the depth of the black layer at that time was 8 cm. Three replicate cores were collected during the daytime high tide and another three cores during daytime low tide for each species. After removal of the high tide cores, the overlying water was collected and fixed with 0.1 ml of 5%

formalin, resulting in an approximate final concentration of 0.5% formalin. These cores, collected with thin walled, plastic coring tubes (1.05 cm diameter), were cut into 1-cm-thick sections. Prior to sectioning, the depth at which the deeper black sediment layer replaced the shallow yellow layer was measured.

Each section of the core sample was placed in a 20-ml glass vial containing 1 ml of 1- μ m filtered seawater and fixed with 0.1 ml of 5% formalin, resulting in a final concentration of 0.45% formalin. Cell densities were estimated from cell suspensions by counting the number of cells containing intact chloroplasts and nuclei in a Palmer-Maloney counting chamber (0.1 ml). Cell suspensions were obtained by vigorously swirling the vials and then removing the overlying suspension after 3–5 s when the sediment settled to the bottom of the vial. Population-weighted mean depths were calculated for each core by multiplying the proportion of the population in each section by the mid-depth of each section and then summing these products. The three population-weighted mean depths were then used to calculate a mean depth for each species during each tidal sampling period.

TIDAL TANK EXPERIMENTS

Outdoor mesocosm experiments were conducted from June through August 1989. Two 1-wk long trials were conducted for each species. Only one species was examined during each week-long trial and each trial was repeated. In each trial the effect of waves and the presence or absence of a subsurface black layer on downward migration was examined. In the treatment examining the effect of a subsurface black layer on *H. virgata*, the depth of the black layer was varied: 3 cm below the sediment surface in trial 1 and 5 mm below the sediment surface in trial 2. The depth of the subsurface black layer in the trials with *E. proxima* was 3 cm in both trials.

Six shallow raceway tanks (4 m long \times 0.8 m wide \times 0.1 m deep holding 220 l of water) equipped with dumping wave-surge buckets (6-l capacity) were used as experimental replicates (Adey and Loveland 1998). Water from the Newport River estuary was supplied by gravity feed from a large outdoor settling tank. Ebb and flood tides were simulated by removing or replacing a standpipe over the drain in each tank. The timing of emersion and submersion was coincident with that in the collection area of each species.

The tanks were filled with sand from an area with no visible microalgal patches but adjacent to a densely populated area. No attempt was made to destroy the natural assemblage of organisms living in the sediment. After removing shell fragments

and other objects larger than 1 cm, the sand was graded along the length of the tanks, rising from a depth of 0 cm under the wave-surge buckets to a depth of 8 cm at the far end of the tanks. This provided a sloped surface for water drainage during ebb tide. All tanks were subjected to tidal changes and waves for 1 d so that sediment packing was roughly equal among them. The flow of seawater into three of the tanks was diverted away from the wave generators to produce three replicate control tanks without wave action.

The flow rate of seawater into the tanks was set at 12 l min⁻¹, resulting in a dumping cycle of 30 s and an initial wave height of 4 cm at the cell inoculation area in the wave tanks. Five seconds after the initial wave, the wave train had an average wavelength of 30 cm and height of 1 cm. Once the tanks were full, the waves produced in the tanks did not break until after they passed over the inoculation areas. Small ripples in the sand were produced in the area where the waves broke, similar to those observed at the collection area in the field. The waves generated in these experiments were similar to those observed in the collection areas during calm days.

E. proxima and *H. virgata* cells were harvested at 0.6 m and 0.24 m above mean low water (MLW), respectively, for inoculation into the tidal tanks by carefully scraping off the top 1 mm of the sand surface after the appearance of cells during ebb tide. The cells were then separated from the sediment by sequential decantation with aliquots of estuary water filtered to 1 μ m. Cell counts were made with a Neubauer phase hemacytometer to estimate the cell density of this extract. This methodology provided monospecific suspensions of *E. proxima* and *H. virgata*. At the time of cell collection, black subsurface sediment was also collected from the intertidal zone.

Prior to cell inoculation the experimental tanks were drained and a 25 cm \times 25 cm square of sediment was excavated from each tank where the sediment depth was 5 cm. Black sediment was layered into this hole in each tank and covered with some of the excavated sand. In the *E. proxima* experiments, a 2-cm black layer was overlaid by a 3-cm yellow layer. This depth was chosen because it corresponded to the thickness of the yellow layer at the time and place of *E. proxima* collection. In the first trial with *H. virgata*, the thickness of the yellow layer was set at 3 cm to allow a comparison with the results of the *E. proxima* experiments. In the second *H. virgata* trial, the thickness of the yellow layer was 5 mm corresponding to its thickness in the *H. virgata* collection area. The total depth of the sediment in the inoculation areas in the tanks was 5 cm in all experiments which roughly corre-

sponded to the depth of maximum penetration for *H. virgata* and *E. proxima* at the times and areas of collection. The depth of the overlying water column was 5 cm during periods of submersion.

In each tank, cells were inoculated into the 25 cm × 25 cm square containing a subsurface black layer and into an adjacent area with no black layer. Inoculation was accomplished by inserting a 4.5 cm diameter lucite tube into the emerged sediment until it contacted the bottom of the tank. An 8-ml aliquot of the cell suspension was then gently pipetted into the tube. The percolation of the suspension through the sand layered between 5×10^5 cells and 1×10^6 cells over a circular area.

The wave and control tanks were maintained under a natural tidal frequency for 1 wk after which the inoculation sites were sampled during daytime high tide. One core was removed from the center of the two inoculation sites (black layer and no black layer) in each tank. A coring tube (1.05 cm diameter, 8 cm length) was pushed into the sediment until it contacted the bottom of the tank. In the cores with a black layer, the thickness of the yellow layer was measured and recorded. The 3-cm-deep column of water overlying each sediment core was collected and fixed with 5% formalin (final concentration of 0.2% formalin) prior to sectioning. Extruded sections of sediment were placed in 20-ml glass vials and fixed with 5% formalin (final concentration of 0.5% formalin).

The exact width of sections was altered between trials to increase the resolution of the vertical distribution profiles at the depths where the microalgae were concentrated. The *E. proxima* cores in trial 1 were sectioned every 1 cm to a depth of 2 cm and then at 5-mm intervals to a depth of 5 cm. In trial 2, the *E. proxima* cores were sectioned at 5-mm intervals to a depth of 3 cm and then at 1-cm intervals to a depth of 5 cm. All *H. virgata* cores in trial 1 were sectioned every 5 mm to a depth of 3 cm, then at 1-cm intervals to a depth of 5 cm. The *H. virgata* cores with a shallow subsurface black layer in trial 2 were sectioned at 2-mm intervals to a depth of 1 cm, 5-mm intervals to a depth of 2 cm, and 1-cm intervals to a depth of 5 cm to ensure adequate resolution. The *H. virgata* cores in trial 2 without a subsurface black layer were sectioned as in trial 1. These minor changes in section widths between trials do not change the statistical analysis since a single weighted mean depth is derived from each core.

To produce the thinner sections (< 5 mm) used in the second *H. virgata* trial, a more precise coring and sectioning device was constructed from a micrometer screw and the sawn-off barrel of a 10-ml disposable plastic syringe (Joint et al. 1982). Sediment cores were collected using the sawn-off sy-

ringe barrel, which was then attached to the micrometer screw to extrude the sections. The extruded sections were collected and fixed as described previously.

The cell number in each section was determined as described for the in situ vertical distribution samples, using a Palmer-Maloney counting chamber. Depth distributions were generated by using the percentage of the total population counted in each section of the core. Since the deeper sections of a core were thicker than those near the surface, the proportion of cells in the deeper sections were corrected by dividing them by the ratio of the thick section volume to the thin section volume. These corrected proportional values were then multiplied by the mid-depth of each section and totalled to produce the population-weighted mean depth for each core. In addition, composite vertical profiles were constructed by calculating the mean and standard error of the percentage of cells within each depth interval. Algal abundance in each section was expressed as a percentage rather than an absolute number because of the variability in total cell number among replicate cores.

STATISTICAL ANALYSIS

Because the two sediment treatments, with and without a subsurface black layer, were paired in the same tanks, they were evaluated in separate statistical analyses due to lack of independence. In each separate analysis, the effect of waves and experimental trial on the mean depth of the population during high tide was evaluated with two-way analysis of variance (ANOVA). Two-way ANOVA was not applied to the *H. virgata* experiments testing the effect of a subsurface black layer due to the different depths of the anoxic zone in the first and second trials. Separate one-way ANOVAs were employed to test the effect of wave motion on *H. virgata* within each trial. Model effects with $\alpha \leq 0.05$ were considered statistically significant. The mean depth data for all experimental treatments, except the *H. virgata* experiment lacking the subsurface black layer, were \log_{10} -transformed prior to analysis to satisfy the assumptions of the ANOVA model. The logarithmic transform was chosen because the standard deviations were proportional to the means (Zar 1996). In addition, Student t-tests were employed to confirm that the mean population density in the cores removed from the wave treatment tanks was not significantly different from those removed from the control tanks in each trial. All ANOVAs were conducted with the GLM procedure of SAS (SAS 1985).

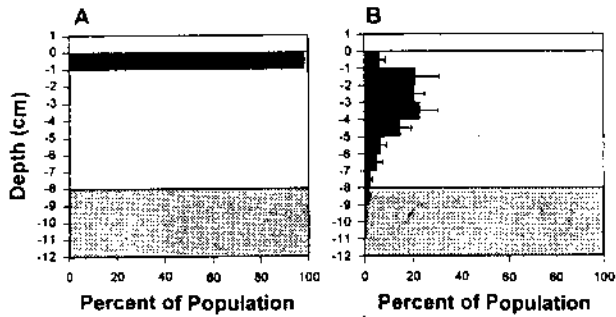


Fig. 1. Vertical profile of *Euglena proxima* under low tide (A) and high tide (B) conditions within an intertidal sand flat on October 17, 1993. The length of each solid horizontal bar is the mean percentage of the total population found in each 1-cm depth interval within three replicate cores. The error bars represent standard errors of the mean. The stippled area indicates the black sediment layer.

Results

IN SITU VERTICAL DISTRIBUTIONS

In the field, *Euglena proxima* migrated from the surface down to the subsurface black layer at a depth of 8 cm during the course of a single tidal cycle (Fig. 1). More than 98% of the *E. proxima* population was no deeper than 1 cm during low tide but under high tide conditions this percentage of the population extended to the 9 cm depth. More than 93% of the population on average was found deeper than 1 cm during high tide. The mean depth of *E. proxima* in situ was 5.9 mm during low tide and 33 mm during high tide. Although *E. proxima* cells were found in the black layer under both high and low tide conditions, these cells represented less than 3% of the total population. Less than 1% on average of the *E. proxima* population was recovered from the water overlying the sediment cores collected during high tide (Fig. 1B).

The *H. virgata* population also demonstrated a dramatic migration response during a single tidal cycle in the field. During low tide, 91% of the *H. virgata* population was located between the sediment surface and a depth of 1 cm (Fig. 2). During high tide over 76% of the *H. virgata* population was located below 1 cm, within the black layer. The mean depth of *H. virgata* in situ was 4.3 mm during low tide and 29 mm during high tide. The lower intertidal position of the *H. virgata* patch is evident from the shallower anoxic zone: 6 mm as compared with 8 cm for the *E. proxima* patch. As with *E. proxima*, less than 1% of the *H. virgata* population was recovered from the water overlying the high tide sediment cores (Fig. 2B).

WAVE TANK EXPERIMENTS

There were no significant effects of wave treatment or experimental trial on mean depth of the

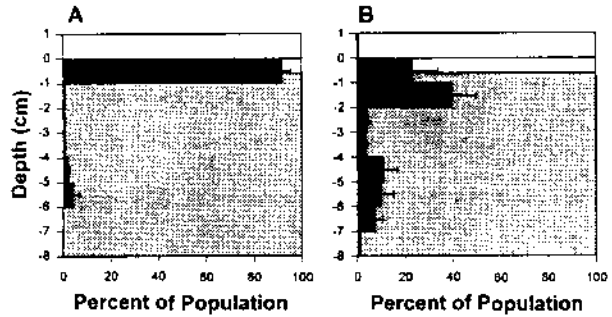


Fig. 2. Vertical profile of the diatom *Hantzschia virgata* var. *intermedia* under low tide (A) and high tide (B) conditions within an intertidal sand flat on July 29, 1993. The length of each solid horizontal bar is the mean percentage of the total population found in each 1-cm depth interval within three replicate cores. The error bars represent standard errors of the mean. The stippled area indicates the black sediment layer.

H. virgata population in the sediment treatment lacking a subsurface black layer (Table 1). The composite profiles reveal that 3–10% of the *H. virgata* population had migrated through the entire sediment column to a depth below 4 cm (Fig. 3).

In the sediment treatment with a subsurface black layer, a downward shift in the vertical position of the *H. virgata* population in the wave tanks relative to the control tanks was apparent in both trials (Fig. 4). However, only trial 1 with the 3-cm-deep black layer resulted in a \log_{10} -transformed mean depth significantly greater than that of the control tanks (Tables 2 and 3). This increase in depth resulting from exposure to waves was not statistically significant in trial 2 where the black layer was originally located at the 5-mm depth (Table 2). At the conclusion of trial 2, the depth of the black layer was still 5 mm in the control tanks but 1 cm in the wave tanks. The mean depth of *H. virgata* in these tanks roughly corresponded to the depth of the black layer (Table 3).

There were significant trial and wave treatment effects on the \log_{10} -transformed mean depth of *E. proxima* in the sediment treatment lacking a subsurface black layer (Table 4). The composite profiles reveal a decrease in the proportion of the population shallower than 1 cm in the wave treatment as compared to the control treatment in trial 1 but

TABLE 1. Results of two-way ANOVA for effect of wave treatment and trial on the mean depth of *Hantzschia virgata* during high tide in the sediment treatment lacking a subsurface black sediment layer.

Source	df	MS	F	p
Trial	1	4.130	0.14	0.715
Wave	1	0.203	0.01	0.935
Trial \times wave	1	30.528	1.06	0.333
Error	8	28.790		

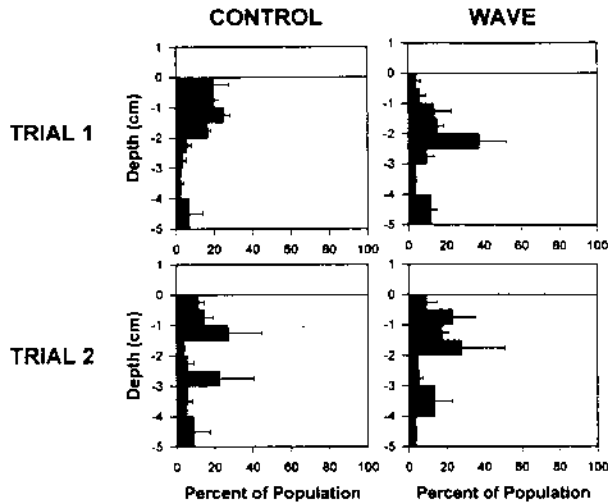


Fig. 3. Composite vertical profiles of *Hantzschia virgata* var. *intermedia* in the sediment treatment lacking a subsurface black layer during high tide in the experimental tidal tanks. The length of each solid horizontal bar is the mean percentage of the total population found in each depth interval within three replicate cores. The error bars represent standard errors of the mean.

this response was not apparent in trial 2 (Fig. 5). In trial 1, the population mean depth of *E. proxima* in the control tanks was about half that of the wave tanks (Table 3).

The results of the analysis of *E. proxima* in the sediment treatment with a subsurface black layer revealed no significant trial or wave treatment effect; however, there was a significant trial \times wave interaction (Table 5). The presence of the subsurface black layer limited the downward migration of *E. proxima*. In the sediment treatment lacking the subsurface black layer, 5–22% of the *E. proxima* population on average was found below 4 cm (Fig. 5); however, in the sediment treatment with a 3 cm deep subsurface black layer, only 0–8% of the *E. proxima* population was found below 4 cm (Fig. 6).

The mean population density of *E. proxima* in the cores removed from the control tanks was not significantly different from those of the wave treatment tanks under either of the sediment treatments or in any of the trials ($p \geq 0.29$). The total number of cells found in the typical core sample for *E. proxima* was 6,000 cells. Similarly, the mean population density of *H. virgata* in the control and wave treatment tanks did not differ under either of the sediment treatments or in any trial ($p \geq 0.10$). The number of cells in the typical *H. proxima* core sample was 2,000.

Discussion

In situ sampling revealed a relatively large vertical distribution for both species: 8 cm for *Hantz-*

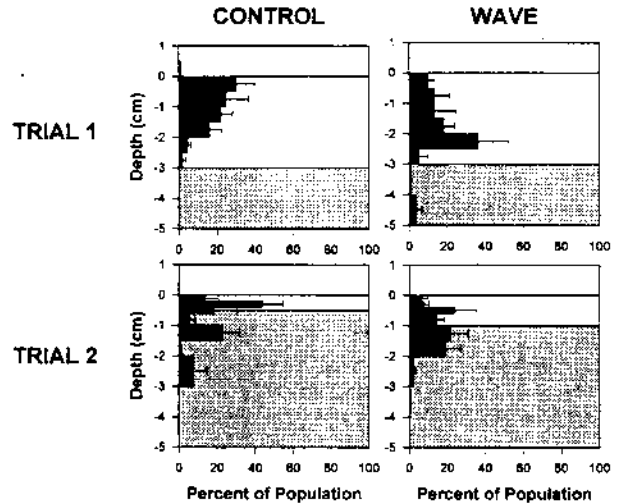


Fig. 4. Composite vertical profiles of *Hantzschia virgata* var. *intermedia* in the sediment treatment with a subsurface black layer during high tide in the experimental tidal tanks. The length of each solid horizontal bar is the mean percentage of the total population found in each depth interval within three replicate cores. The error bars represent standard errors of the mean. The stippled area indicates the black layer.

schia virgata and up to 12 cm for *Euglena proxima*. Earlier studies conducted on intertidal mud and sand flats suggest the vertical distributions of photosynthetic microalgae do not exceed 3 cm in depth (Fenchel and Reidl 1970; Elliott and Bamforth 1975). Much of the literature concerning vertical migration of benthic microalgae has centered on species inhabiting mud flats, where the amplitude of migration is generally 3 mm for diatoms (Aleem 1950; Hopkins 1963, 1966a; Round and Palmer 1966; Round 1978, 1979; Paterson 1986; Pinckney et al. 1994) and euglenoids (Palmer and Round 1965). One study that did examine a sand flat community found that four diatom species migrated down to 1 cm and one blue-green alga migrated as far down as 2 cm (Joint et al. 1982). Sev-

TABLE 2. Results of one-way ANOVA for effect of wave treatment on the \log_{10} transformation of mean depth of *Hantzschia virgata* during high tide in the sediment treatment with a subsurface black layer. The depth of the interface between the black layer and the overlying sediment was 3 cm in trial 1 and 1 cm in trial 2 at the end of the experiment. The asterisk denotes a significant treatment effect at $\alpha = 0.05$.

Source	df	MS	F	p
Trial 1				
Wave	1	0.4424	7.67	0.050*
Error	4	0.0577		
Trial 2				
Wave	1	0.0555	4.21	0.110
Error	4	0.0132		

TABLE 3. Weighted mean depths of *Hantzschia virgata* and *Euglena proxima* populations at high tide ± 1 SE in control and wave tanks after trials 1 and 2. Values are expressed in cm. Each mean and SE was calculated from three replicate samples. The depth of the black layer was 3 cm except for the second trial involving *Hantzschia virgata*; at the end of this trial the depth of the black layer was 5 mm in the control tanks and 1 cm in the wave tanks.

Sediment Treatment	Trial	<i>Hantzschia virgata</i>		<i>Euglena proxima</i>	
		Wave	Control	Wave	Control
Without subsurface black layer	1	1.79 \pm 0.27	1.44 \pm 0.33	2.81 \pm 0.57	1.54 \pm 0.19
	2	1.59 \pm 0.14	1.88 \pm 0.42	2.82 \pm 0.09	2.73 \pm 0.29
With subsurface black layer	1	1.69 \pm 0.24	0.98 \pm 0.13	2.77 \pm 0.06	2.54 \pm 0.20
	2	0.95 \pm 0.17	0.62 \pm 0.20	2.39 \pm 0.16	2.85 \pm 0.12

eral other studies concluded that most of the living diatoms on sand flats occur in the uppermost centimeter of sand (Meadows and Anderson 1968; Harper 1969; Riznyk and Phinney 1972).

The deeper in situ vertical distributions reported for *H. virgata* and *E. proxima* in this study are probably related to time- and site-specific factors such as wave exposure, sediment grain size, and depth of the yellow-black sediment interface. On all mud flats and many sand flats, the depth of the black sediment layer is relatively shallow, especially in mid to low intertidal areas. However, the high intertidal location of the *E. proxima* patch during October 1993 resulted in a 8 cm deep black layer. The depth of the yellow-black sediment interface in the upper intertidal areas where *E. proxima* was found ranged from 3 cm to 10 cm in depth depending upon season and location (Kingston 1990).

The mesocosm experiments in the tidal tanks revealed that the vertical distribution of both species was affected by waves under certain conditions. The mean depth of populations exposed to waves was sometimes deeper than that of populations exposed to calm water conditions; however, the significance of the wave response was mediated by the presence and depth of the subsurface black layer. In *H. virgata*, the effect of wave action on vertical migration was not detected with the yellow-black layer interface at 5 mm, but the wave effect was statistically significant with the interface at 3 cm. In *E. proxima*, the effect of wave action was only detected in the absence of a subsurface anoxic layer. Consequently, the vertical position of each species is governed by some interplay between oppos-

ing vertical gradients—wave energy decreasing with depth, anoxia and reducing conditions increasing with depth.

The chosen response variable in the mesocosm experiments was the mean depth of the population at high tide. A change in the mean depth of the population can result from two separate processes. Each cell in a population can respond to wave-induced sediment disturbance by migrating deeper. Alternatively, individual cells that migrate to shallower depths can be removed by waves, resulting in an increased mean depth of the population even though there is no change in the behavior of individual cells. The results of the Student's *t*-tests revealed that the total number of cells in cores removed from control tanks was not significantly different from that in cores removed from wave tanks. Consequently, the wave energy used in this study did not result in the removal of a significant proportion of the population by suspension in the water column. This suggests that individual cells re-

TABLE 4. Results of two-way ANOVA for effect of wave treatment and trial on \log_{10} -transformed mean depth of *Euglena proxima* during high tide in the sediment treatment lacking a subsurface black sediment layer. The asterisk denotes a significant treatment effect at $\alpha = 0.05$.

Source	df	MS	F	p
Trial	1	0.0535	5.93	0.041*
Wave	1	0.0542	6.00	0.040*
Trial \times wave	1	0.0402	4.46	0.068
Error	8	0.0009		

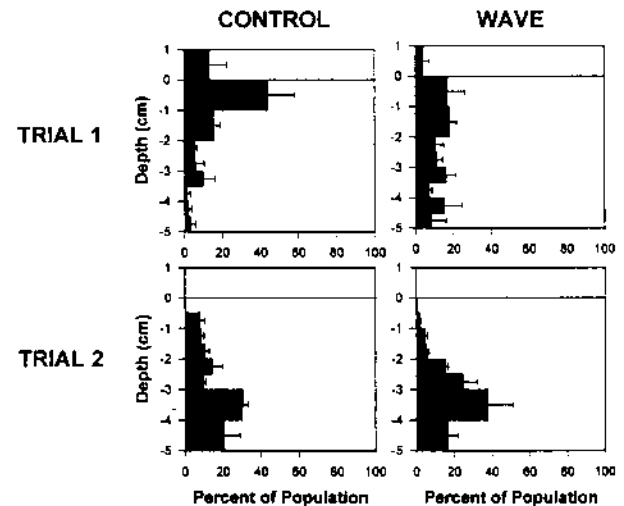


Fig. 5. Composite vertical profiles of *Euglena proxima* in the sediment treatment lacking a subsurface black layer during high tide in the experimental tidal tanks. The length of each solid horizontal bar is the mean percentage of the total population found in each depth interval within three replicate cores. The error bars represent standard errors of the mean.

TABLE 5. Results of two-way ANOVA for effect of wave treatment and trial on \log_{10} -transformed mean depth of *Euglena proxima* during high tide in the sediment treatment with the interface between the black layer and the overlying sediment at a depth of 3 cm. The asterisk denotes a significant treatment effect at $\alpha = 0.05$.

Source	df	MS	F	p
Trial	1	0.0001	0.08	0.787
Wave	1	0.0010	0.59	0.463
Trial \times wave	1	0.0103	4.46	0.041*
Error	8	0.0017		

sponded to the wave treatment by migrating deeper into the sediment.

The absence of a significant wave effect for *H. virgata* populations in the sediment treatment lacking a subsurface black layer may involve the role of the black layer in slowing downward migration. Without a subsurface black layer to slow downward migration, the cells may continue their descent throughout the high tide period rather than migrating to a specific depth and remaining there until the next tide change. A comparison of the vertical profiles in Figs. 3 and 4 shows that the cells were dispersed over a greater range of depths in the sediment treatment lacking a subsurface black layer than in the sediment treatment with a subsurface black layer. The greater dispersion of the populations in this sediment treatment would increase the variance around the mean depth and mask any wave effects. The absence of a wave effect in this sediment treatment for *H. virgata* may well be due to the absence of a subsurface black layer to act as a signal to slow downward migration.

The wave treatment did result in a significantly deeper mean depth for *H. virgata* in the presence of a subsurface black layer but only when the yellow-black sediment interface was deeper (3 cm). It appears that a 3 cm deep yellow sediment layer was deep enough to resolve differences in the mean depth of the populations exposed to wave and control conditions. The lack of a wave effect with a 5 mm deep black layer may have been due to inadequate vertical sampling resolution. The situation is complicated further by the fact that the wave motion deepened the anoxic interface to 1 cm in the wave treatment while it remained at 5 mm in the control treatment.

The results of the present study suggest that the yellow-black sediment interface was not a hard boundary limiting downward migration of *H. virgata* since many cells were found in the black layer in the field and in the experimental tanks. Fenchel and Riedl (1970) also observed great numbers of some diatoms, euglenoids, and dinoflagellates within the black sediment layer. The mean depth of *H. virgata* in the control and wave tanks roughly

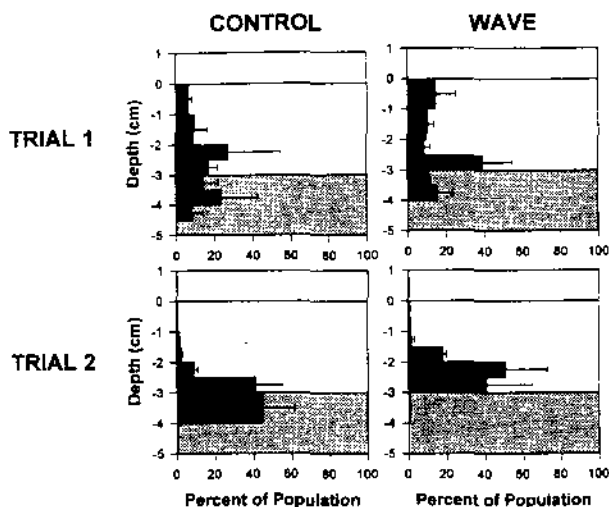


Fig. 6. Composite vertical profiles of *Euglena proxima* in the sediment treatment with a subsurface black layer during high tide in the experimental tidal tanks. The length of each solid horizontal bar is the mean percentage of the total population found in each depth interval within three replicate cores. The error bars represent standard errors of the mean. The stippled area indicates the black layer.

corresponded to the depth of the yellow-black sediment interface. This suggests that this interface, which is roughly correlated with an increase in hypoxia and reducing conditions, may serve as a depth regulator that loosely controls the amplitude of vertical migration of *H. virgata*. *H. virgata* may be similar to the diatom *Surirella gemma*, which uses positive aerotaxis to slow downward migration (Hopkins 1966a).

The mean depth of *E. proxima* was significantly increased by wave action in the sediment treatment lacking a subsurface black layer. In addition to the significant wave effect, the two-way ANOVA revealed a significant trial effect. This difference between trials has no simple explanation. The trial effect may be due to differences in the recent history of the cells prior to collection and inoculation, or differences in other environmental conditions during each trial. The amount of rainfall was four times greater and the average wind speed three times greater during the second *E. proxima* trial than during the first (Kingston 1990). The downward migration in the control tanks during trial 2 rivaled that in the wave tanks. It is possible that the greater downward migration observed in the control tanks of trial 2 relative to trial 1 was a response to a change in salinity, physical disturbance by rain drops, or increased wind shear on the overlying water.

The mean depth of *E. proxima* did not show a significant wave or trial effect in the experiments with a subsurface black layer; however, the wave \times

trial interaction was significant. This interaction effect indicates a reversal in the effect of the wave treatment between trials. This reversal may be related to the environmental differences between trials 1 and 2 described above. Despite the differences between trials, it is obvious that the presence of a subsurface black layer at 3 cm obscured the significant wave effect observed in the sediment treatment lacking the black layer.

Concentrating on trial 1, which was not affected by rainstorms, it is apparent that the mean depth of the cells in the control tanks was greater with a subsurface black layer present than in its absence (Table 3). This means that *E. proxima* cells located in the same tanks and exposed to identical water conditions migrated to greater depths if they were located above a subsurface black layer. Consequently, the absence of a significant wave effect in the sediment treatment with a black layer may have been due to the overriding effect of the black sediment layer. This suggests that the mean depth of *E. proxima* during high tide was more tightly controlled by the depth of the black layer than by wave energy. The shallower depth of *E. proxima* populations in the treatments lacking a subsurface black layer may be due to the absence of a directional chemical gradient produced by the yellow-black interface.

Why would a subsurface anoxic layer reinforce a stronger downward migration response in *E. proxima*? One explanation is that the yellow-black interface represents a reliable indicator of the depth of sediment mixing. The depth of the yellow-black interface is not just the product of instantaneous wave action but incorporates information about the wave history of a location over a much longer time scale. Consequently, individuals that used the depth of the interface as an indicator of migration distance may have gained a selective advantage over others. Through evolutionary time, this trait would be selected for and eventually become fixed in the population. Selection for this trait should not be expected in all species since other adaptations such as agglutination of the sediment can reduce losses to tidal suspension (Hopkins 1964; Paterson 1989; Paterson et al. 1990). Fauré-Fremiet (1951) noted that subsurface aggregations of *H. virgata* secrete sediment-agglutinating mucus, which provides stability.

Another explanation for the migration behavior of *E. proxima* is that migration to the black layer provides some benefit, such as access to nutrient-enriched porewater. Tidal tank experiments revealed that the mean depth of *E. proxima* populations during high tide is significantly deeper in containers in which the porewater at the bottom of the containers is enriched with ammonium, ni-

trate, phosphate, glucose, and acetate than in control containers lacking the added nutrients (Kingston 1990). These results suggest that the presence of an enriched pool of nutrients below the sediment surface reinforces the downward migration of *E. proxima* during high tide.

Previous studies have verified that several nutrients increase with depth in coastal sediments, including amino acids (Henrichs and Farrington 1979), dissolved inorganic carbon (Fenchel and Riedl 1970; Howes et al. 1985), and ammonium (Fenchel and Riedl 1970; Kenworthy et al. 1982). Several authors suggest downward migration of benthic microalgae provides access to subsurface nutrients that may support growth during conditions of nutrient depletion in the overlying waters (Admiraal 1984; Happey-Wood and Priddle 1984; Happey-Wood and Jones 1988).

The present study provides the first experimental evidence that wave energy and the depth of the yellow-black sediment interface influence the vertical migration of benthic microalgae. For both species studied, the yellow-black interface limited the extent of downward migration but did not provide a barrier to migration. The response of *H. virgata* to increased wave energy may have been limited by the depth of the yellow-black interface since a significant wave effect was detected when the anoxic layer was 3 cm deep but not 5 mm deep. A significant wave effect was detected in *E. proxima* in the absence of a subsurface black layer. This wave effect was not observed when a subsurface black layer was present, possibly due to undetermined attractant properties associated with this layer, such as greater nutrient availability. These findings lend support for the idea that downward migration provides an avenue of escape from waves that would physically displace benthic microalgae from their intertidal habitat. Future experiments including vertical profiles of oxygen and redox potential may help to elucidate the effect of the interaction between waves and the depth of the black layer on microalgal vertical migration.

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LITERATURE CITED

- ADEY, W. H. AND K. LOVELAND. 1998. *Dynamic Aquaria: Building Living Ecosystems*, 2nd ed., Academic Press, New York.

- ADMIRAAL, W. 1984. The ecology of estuarine sediment-inhabiting diatoms, p. 269-322. In F. E. Round and D. J. Chapman (eds.), *Progress in Phycological Research*, Vol. 3, Biopress Ltd, Bristol, United Kingdom.
- ADMIRAAL, W. AND H. PELETIER. 1979. Sulfide tolerance of benthic diatoms in relation to their distribution in an estuary. *British Phycological Journal* 14:185-196.
- ALEEM, A. A. 1950. The diatom community inhabiting the mud flats at Whitstable. *New Phytologist* 9:174-188.
- ANDERSON, V., J. SARDOU, AND P. NIVAL. 1992. The diel migrations and vertical distribution of zooplankton and micronekton in the northwestern Mediterranean Sea. 2. Siphonophores, hydromedusae and pyrosomids. *Journal of Plankton Research* 14:1155-1169.
- ANGEL, M. V. 1985. Vertical migrations in the oceanic realm: Possible causes and probable effects. *Contributions in Marine Science, University of Texas* 27(Suppl.):45-70.
- ANGEL, M. V. 1989. Vertical profiles of pelagic communities in the vicinity of the Azores Front and their implications to deep ocean ecology. *Progress in Oceanography* 22:1-46.
- BAILLIE, P. W. AND B. L. WELSH. 1980. The effect of tidal resuspension on the distribution of intertidal epipelagic algae in an estuary. *Estuarine, Coastal and Shelf Science* 10:165-180.
- BOLLENS, S. M. AND B. W. FROST. 1991. Diel vertical migration in zooplankton: Rapid individual response to predators. *Journal of Plankton Research* 13:1359-1365.
- CALLAME, B. AND J. DEBYSER. 1954. Observations sur les mouvements des diatomées à la surface des sédiments marins de la zone intercotidale. *Vie et Milieu* 5:242-249.
- CONNOR, M. S. 1980. Snail grazing effects on the composition and metabolism of benthic diatom communities and subsequent effects on fish growth. Ph.D. thesis, Massachusetts Institute of Technology and Woods Hole Oceanographic Institution (WHOI-80-26), Cambridge, Massachusetts.
- CONNOR, M. S., J. M. TEAL, AND I. VALIELA. 1982. The effects of feeding by mud snails, *Ilyanassa obsoleta* (Say), on the structure and metabolism of a benthic algal community. *Journal of Experimental Marine Biology and Ecology* 65:29-45.
- ELLIOTT, P. B. AND S. S. BAMFORTH. 1975. Interstitial protozoa and algae of Louisiana salt marshes. *Journal of Protozoology* 22:514-519.
- FAURÉ-FREMIET, E. 1951. The tidal rhythm of the diatom *Hantzschia amphioxys*. *Biological Bulletin* 100:173-177.
- FALVEL, P. AND G. BOHN. 1907. Le rythme des marées chez les diatomées littorales. *Compte Rendu des Séances de la Société Biologique* 62:121-123.
- FENCHEL T. AND L. H. KOFOED. 1976. Evidence for exploitative interspecific competition in mud snails (Hydrobiidae). *Oikos* 27:367-376.
- FENCHEL, T. AND R. J. RIEDL. 1970. The sulfide system: A new biotic community underneath the oxidized layer of marine sand bottoms. *Marine Biology* 7:255-268.
- FORWARD, R. B., JR. 1976. Light and diurnal vertical migration: Photobehavior and photophysiology of plankton, p. 157-209. In K. C. Smith (ed.), *Photochemical and Photobiological Reviews*, Volume 1, Plenum Press, New York.
- FORWARD, R. B., JR. 1988. Diel vertical migration: Zooplankton photobiology and behavior. *Oceanography and Marine Biology Annual Review* 26:361-393.
- GANAPATI, P. N., M. V. LAKSHMANA RAO, AND D. V. SUBBA RAO. 1959. Tidal rhythms of some diatoms and dinoflagellates inhabiting the intertidal sands of Visakhapatnam Beach. *Current Science* 11:450-451.
- GLIWICZ, M. Z. 1986. Predation and the evolution of vertical migration in zooplankton. *Nature* 320:746-748.
- HAPPEY-WOOD, C. M. AND P. JONES. 1988. Rhythms of vertical migration and motility in intertidal benthic diatoms with particular reference to *Pleurosigma angulatum*. *Diatom Research* 3:83-93.
- HAPPEY-WOOD, C. M. AND J. PRIDDLE. 1984. The ecology of epipelagic algae in five Welsh lakes, with special reference to Volvoclean green flagellates. *Journal of Phycology* 20:109-124.
- HARPER, M. A. 1969. Movement and migrations of diatoms on sand grains. *British Phycological Journal* 4:97-103.
- HECKMAN, S. M. 1985. The development of vertical migration patterns in the sediments of estuaries as a strategy for algae to resist drift with tidal currents. *Internationale Revue der Gesamten Hydrobiologie* 70:151-164.
- HENRICHS, S. M. AND J. W. FARRINGTON. 1979. Amino acids in interstitial waters of marine sediments. *Nature* 279:319-322.
- HOPKINS, J. T. 1963. A study of the diatoms of the Ouse Estuary, Sussex-I. The movement of the mud-flat diatoms in response to some chemical and physical changes. *Journal of the Marine Biological Association of the United Kingdom* 43:653-663.
- HOPKINS, J. T. 1964. A study of the diatoms of the Ouse Estuary, Sussex-II. The ecology of the mud-flat diatom flora. *Journal of the Marine Biological Association of the United Kingdom* 44:333-341.
- HOPKINS, J. T. 1966a. Some light-induced changes in behaviour and cytology of an estuarine mud-flat diatom, p. 335-358. In R. Bainbridge (ed.), *Light as an Ecological Factor*, Blackwell, Oxford.
- HOPKINS, J. T. 1966b. The role of water in the behavior of an estuarine mud-flat diatom. *Journal of the Marine Biological Association of the United Kingdom* 46:617-626.
- HOWES, B. L., J. W. H. DACEY, AND S. G. WAKEHAM. 1985. Effects of sampling technique on measurements of porewater constituents in salt marsh sediments. *Limnology and Oceanography* 30:221-227.
- JOINT, I. R., J. M. GEE, AND R. M. WARWICK. 1982. Determination of fine-scale vertical distribution of microbes and meiofauna in an intertidal sediment. *Marine Biology* 72:157-164.
- KENWORTHY, W. J., J. C. ZIEMAN, AND G. W. THAYER. 1982. Evidence for the influence of seagrasses on the benthic nitrogen cycle in a coastal plain estuary near Beaufort, North Carolina (USA). *Oecologia* 54:152-158.
- KINGSTON, M. B. 1990. Vertical migration of benthic microalgae. Ph.D. thesis, Duke University, Durham, North Carolina.
- MEADOWS, P. S. AND J. G. ANDERSON. 1968. Microorganisms attached to marine sand grains. *Journal of the Marine Biological Association of the United Kingdom* 48:161-175.
- NEILSON, J. D. AND R. I. PERRY. 1990. Diel vertical migrations of marine fishes: An obligate or facultative process? *In Advances in Marine Biology* 26:115-168.
- PALMER, J. D. 1976. Clock-controlled vertical migration rhythms in intertidal organisms, p. 239-255. In P. J. Coursey (ed.), *Biological Rhythms in the Marine Environment*, University of South Carolina Press, Columbia, South Carolina.
- PALMER, J. D. AND F. E. ROUND. 1965. Persistent, vertical-migration rhythms in benthic microflora I. The effect of light and temperature on the rhythmic behaviour of *Euglena obtusa*. *Journal of the Marine Biological Association of the United Kingdom* 45:567-582.
- PALMER, J. D. AND F. E. ROUND. 1967. Persistent, vertical-migration rhythms in benthic microflora. VI. The tidal and diurnal nature of the rhythm in the diatom *Hantzschia virgata*. *Biological Bulletin* 132:44-55.
- PATERSON, D. M. 1986. The migratory behaviour of diatom assemblages in a laboratory tidal micro-ecosystem examined by low temperature scanning electron microscopy. *Diatom Research* 1:227-239.
- PATERSON, D. M. 1989. Short-term changes in the erodibility of intertidal cohesive sediments related to the migratory behaviour of epipelagic diatoms. *Limnology and Oceanography* 34:223-234.
- PATERSON, D. M., R. M. CRAWFORD, AND C. LITTLE. 1990. Sub-aerial exposure and changes in the stability of intertidal es-

CHANGES IN THE VERTICAL DISTRIBUTION OF *Amphora ovalis* BIOMASS IN INTERTIDAL MUDDY SEDIMENTS. *Diatom Research* 9:143-153.

REISE, K. 1985. Tidal Flat Ecology: An Experimental Approach to Species Interactions. Springer-Verlag, New York.

RIZNIK, R. Z. AND H. K. PHINNEY. 1972. Distribution of intertidal phytoplankton in an Oregon estuary. *Marine Biology* 13:318-324.

ROUND, F. E. 1978. On rhythmic movement of the diatom *Amphora ovalis*. *British Phycological Journal* 13:311.

ROUND, F. E. 1979. Occurrence and rhythmic behaviour of *Tri-*

triatum in the intertidal zone. *Journal of Marine Research* 37:241-244.

SAS. 1985. SAS/STAT Guide for Personal Computers. Version 5 edition. SAS Institute, Cary, North Carolina.

TAYLOR, W. R. 1967. The occurrence of *Euglena deses* on the sands of Sierra Leone peninsula. *Journal of Ecology* 55:345-359.

ZAR, J. H. 1996. Biostatistical Analysis, 3rd edition. Prentice Hall, Upper Saddle River, New Jersey.

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